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Effects of Stratification, Warm Treatment, and Mechanical and Acid Scarification on the Emergence of Yellow-Flowered Alfalfa (Medicago sativa subsp. falcata) Seeds

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Effects of Stratification, Warm Treatment, and Mechanical and Acid Scarification on the Emergence of Yellow-Flowered Alfalfa *(Medicago sativa subsp. falcata)* Seeds

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ABSTRACT

Yellow-flowered alfalfa (YFA) has great potential to improve rangelands in the Northern Plains. Greater than 99% of YFA seeds from soil were viable but less than 4% germinated under standard laboratory conditions. The objective was to determine if low germination was due to physical or physiological seed dormancy. The experiment utilized a random complete block design with two trials, eight replications per trial. Each replication consisted of ten treatments plus a control, four stratification treatments consisting of a combination of two temperatures (-5°C, -20°C) and two durations (48 hr, 72 hr), one warm treatment (4 min, 60°C), four sandpaper scarification treatments with one grade (220) and four durations (2 sec, 4 sec, 6 sec, 8 sec), and one 17-18 M sulfuric acid treatment with duration (30 sec trial 1 and 25 min trial 2). Seeds were planted at the same time in plastic trays filled with potting soil and maintained in a growth room at constant temperature. Tray water content was maintained daily by misting. Number of seedlings, light intensity and temperature were recorded daily for seven days and then every third day for 28 days. Two-sample t-test was used to determine the sandpaper scarification treatments significantly increased the final emergence (88%) compared to control (28%) in both trials. Sulfuric acid had a greater final emergence than control. As the duration of the acid treatment increased from 30 sec to 25 min, the emergence increased from 37% to 76%. Results from stratification treatments varied between two trials. There was no significant difference between warm treatment and control for both trials.

Keywords: *Medicago sativa subsp. falcata*, yellow-flowered alfalfa, legume, emergence, hard seed, scarification, stratification

INTRODUCTION

Although alfalfa has been planted on millions of acres and more than 100 varieties have been developed over the past 100 years in North America since its introduction, incidences of alfalfa becoming naturalized in North American rangelands are extremely rare (Rumbaugh 1982). However, recently it was discovered that a population of predominantly

yellow-flowered alfalfa (YFA) (*Medicago sativa subsp. falcata*) has become naturalized on private and adjacent public rangeland in northwestern South Dakota (Smith 1997, Xu et al. 2004). YFA, a legume native to the Middle East and Central Asia, was introduced to western South Dakota by N. E. Hansen at the turn of the 20th century from Siberia (Rumbaugh, 1979). YFA has a natural range of adaptation up to 64°N in Siberia where climatic conditions are comparable to the northern Great Plains of the United States of America (Hansen 1909). YFA is more winter-hardy, more drought tolerant, and grazing tolerant than other alfalfas due to its deep-set crown and fibrous root systems (Berdahl et al. 1989). It has been proven to be more persistent in rangelands in the Northern Great Plains (Berdahl et al. 1989). The occurrence of a population with the ability to reproduce naturally in native rangeland points strongly to its value for rehabilitating millions of hectares of severely depleted rangelands worldwide. Previous research and/or producer observations have shown the benefits of interseeding of YFA into a native mixed-grass rangeland that included increase forage production and quality, nitrogen fixation, carbon sequestration, carrying capacity and animal production, and improved wildlife habitat (Mortenson et al, 2004, 2005, Smith 1997, Boe et al. 1998). It shows the potential to increase long-term sustainability of the ecosystem.

While naturalized YFA populations and seed bank exist (Xu et al. 2004, 2008), the seedling survival of YFA is not high (Rumbaugh 1982). The YFA seedling recruitment and establishment success rate under natural condition are presently unknown. Results from preliminary study demonstrated that greater than 99% of YFA seeds in the soil seed bank were viable but less than 4% germinated under standard laboratory conditions (Xu et al. 2008). The lack of understanding about what factors (physiological dormancy vs. physical dormancy) inhibit the germination is a critical deficiency. Until this information becomes available, it will be difficult to properly utilize YFA in the semiarid rangelands. Being part of the legume family, alfalfa has the hard seed coat characteristics that prevents germination by keeping moisture from entering (Kinch et al. 1914). This study was designed to test two hypotheses. The first hypothesis was to test whether the germination was inhibited by physical dormancy which could be improved by methods of scarification. The second hypothesis was to test whether the germination was inhibited by physiological dormancy which can be improved by methods of stratification. The objectives of the study were to determine: 1) the effects of mechanical and acid scarification on the seedling emergence of YFA and 2) the effects of stratification and warm treatment on the seedling emergence of YFA.

METHODS

Seed Source

The seeds used for this study were hand harvested from a naturalized population of alfalfa in the Grand River National Grassland (45º49’N, 102º33’W), South Dakota. It was important to use hand harvested seeds because mechanical harvesters could pre-scarify seeds. About 10,000 intact seeds were carefully selected by hand under a 4x magnifier on a light table for the study.

Scarification Treatments

There were five scarification treatments that tested the physical dormancy hypothesis and consisted of four sandpaper treatments and one acid treatment. A Forsberg scarifier lined with a sheet of grade 220 sandpaper was used to scarify the seeds for four durations of 2, 4, 6 and 8 seconds. For acid treatment, seeds were soaked in 17-18 M sulfuric acid for 30 seconds (Muir and Pitman, 1987) in trial one. Due to results from blotter germination tests conducted between the two trials, the acid treatment duration was increased to 25 minutes for the second trial.

Stratification Treatments

There were four cold stratification treatments consisting of a combination of two temperatures (-5°C, -20°C) and two durations (48 hr, 72 hr). The seeds were double bagged to prevent moisture loss and then put into freezers. There was one warm treatment where the seeds were placed in a dry oven at 60°C for 4 minutes which is comparable to the time that a grassland fire would burn through (Martin et al. 2975, Cushwa et. al, 1968).

Experimental Design

The experiment utilized a random complete block design with two trials, eight replications per trial. Each block was a 54 cm x 27 cm x 6.5 cm tray filled with Pro-Mix peat/bark growing medium. There were 11 rows per block, each row being a treatment, including one control row. Fifty seeds were planted per row to a depth of 1.0 to 1.5 cm.

The seeds were planted at the same time and kept in a growth room on a 12 hr day/night cycle. Light intensity was maintained between 35-84 μmolms and temperature was maintained between 20°C-25°C. The trays were maintained at maximum water capacity daily by misting. The locations of trays were rotated within two shelves daily to prevent microenvironments from developing.

Data collection and analysis

An emerged seed was considered when the cotyledon emerged through the growing medium surface. The number of emerged seeds, light intensity and temperature were recorded daily for seven days and every third day for 28 days total for each trial. The percent daily emergence rates were calculated and graphed. A two-sample t-test was used to calculate the significance of the final emergence of each treatment compared to the control at $\alpha=0.05$.

RESULTS

The scarification treatments significantly ($P< 0.05$) increased the final emergence of YFA seeds compared to the control in both trials (Fig. 1, Fig. 2). The final emergence of control treatment varied between 26% to 28% from the trial two to the trial one. The final emergence of the acid treatment was significantly higher than the control treatment ($P< 0.05$); 39% of seedlings emerged in trial one and 76% in trial two. For the sandpaper scarification treatments the final emergence ranged from 82 to 90% with a mean of 88% for trial one and 50 to 91% with a mean of 79% for trial two.

The daily emergence rates of the physical scarification treatments were higher than that of the control. In trial one the average daily emergence rate of the four sandpaper treatments varied between 41% and 47% while the acid scarification treatment increased the rate to 19% (Fig. 1). In trial two the daily average emergence rates showed an increase for the sandpaper treatment of 35% to 71% compared to the control (16%). The acid scarification treatment for trial two produced a high emergence rate of 63% (Fig.2).

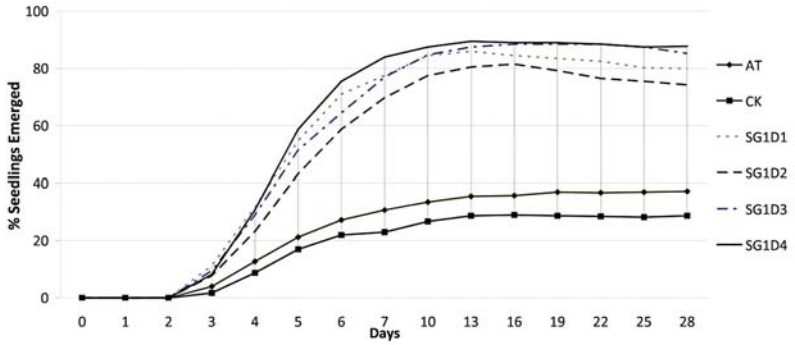

Figure 1. Percent seedling emergence of YFA subject to physical treatments AT (acid treatment for 30 seconds in 17-18 M sulfuric acid), SG1D1 (sandpaper scarification by grade 220 sandpaper for 2 seconds), SG1D2 (sandpaper scarification by grade 220 sandpaper for 4 seconds), SG1D3 (sandpaper scarfication by grade 220 sandpaper for 6 seconds), SG1D4 (sandpaper scarfication by grade 220 sandpaper for 8 seconds) and CK (control) for trial one.

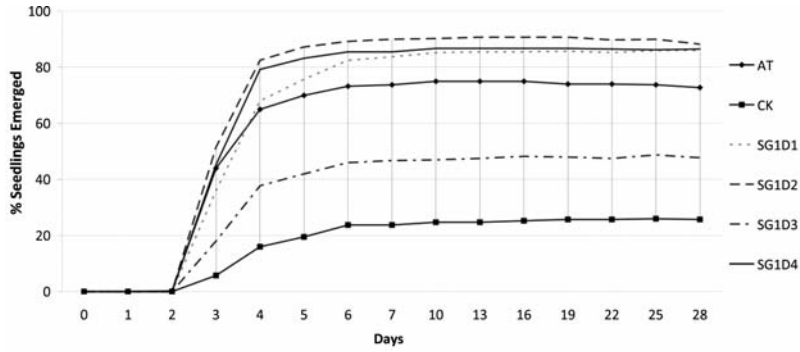

Figure. 2. Percent seedling emergence of YFA subject to physical treatments AT (acid treatment for 25 minutes in 17-18 M sulfuric acid), SG1D1 (sandpaper scarification by grade 220 sandpaper for 2 seconds), SG1D2 (sandpaper scarification by grade 220 sandpaper for 4 seconds), SG1D3 (sandpaper scarfication by grade 220 sandpaper for 6 seconds), SG1D4 (sandpaper scarfication by grade 220 sandpaper for 8 seconds) and CK (control) for trial two.

The results of the stratification and warm treatments on the final emergence varied between trials. There was no difference detected between the treatments and the control in trial one ($P>0.05$). However, on trial two the stratification treatments of -20°C for 48 hrs and 72 hrs significantly ($P<0.05$) increased the final emergence (43%) compared to the control (26%) (Fig. 3).

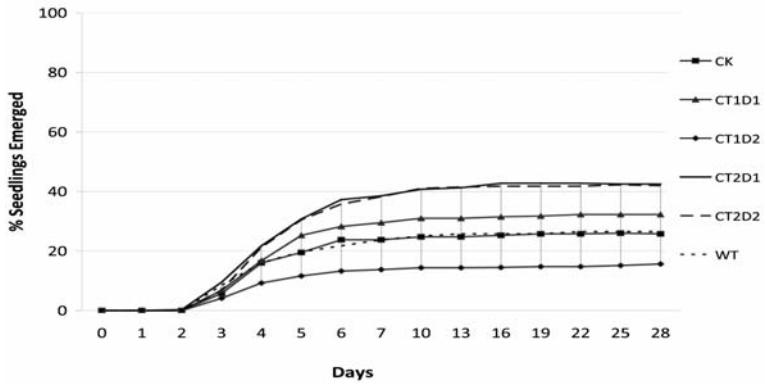

Figure 3. Percent seedling emergence of physiological treatments CT1D1 (stratification at temperature -5°C for 48 hrs), CT1D2 (stratification at temperature -5°C for 72 hrs), CT2D1 (stratification at -20°C for 48 hrs), CT2D2 (stratification at -20°C for 72 hrs) and CK (control) for trial two.

DISCUSSION

The results clearly demonstrate that the most effective method of improving the seedling emergence of YFA proved to be scarification, particularly sandpaper treatments. This indicates that the low emergence of YFA is probably due to physical dormancy which was consistent with the results from previous research done on the legume species *Galactia elliottii* (Muir and Pitman, 1987). Acid treatments significantly increased the seedling emergence of YFA although there was a dramatic difference between the two trials. Seeds that were soaked in the acid for 30 seconds showed an emergence increase to 37% while seeds that were soaked for 25 minutes had an emergence rate of 76%. This suggests that there was a positive correlation between the duration of acid treatment and seedling emergence rate up until the acid breaks through the seed coat and begins destroying the embryo of the seeds. Similar results were reported for other legume species (Cushwa et al. 1968). Emergence was enhanced by scarification, possibly by increasing the permeability to water and gases (Cushwa et al. 1968).

The stratification treatments did not show a consistent response indicating that the low seedling emergence was probably not due to physiological dormancy. These results support the findings of previous research on the freezing and thawing of other leguminous seeds

which reported that freezing seeds to -20°C had little to no effect on germination (Busse, 1930). However, colder temperatures and longer durations have shown to increase germination in sweet clover (*Melilotus sp.*) and purple-flowered alfalfa (*Medicago sativa*) seeds (Busse, 1930). Further research could be done experimenting with different cold temperatures and durations.

Although the warm treatment had no effect on emergence in this study, a previous study examining the effects of moist heat and dry heat on leguminous plants showed that dormancy breaking temperatures ranged from 45°C to 80°C and 45°C to 90°C, respectively (Martin et. al, 1975) so further experimentation with treatments at different temperatures might produce different results. However, Martin and his colleagues (1975) concluded that dry or moist heat exposure 4 min in the range of 90°C to 110°C was lethal to all seed they tested.

The practical applications of this study would be to use the results to determine a pre-treatment for YFA seeds that would help in their establishment success when interseeded in rangelands and pastures.

LIMITATIONS

This study was conducted through the course of one summer, three months, so the main limitation of the study was time constraint. If the study was to have two trials with 28 day growth periods none of the treatments could take more than a few days to complete. This prevented the use of stratification treatments with long durations.

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